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Efficacy of diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in rough rice: Impacts of temperature and relative humidity ☆

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Abstract

Laboratory trials were conducted to determine the effectiveness of diatomaceous earth (DE) against *Rhyzopertha dominica* (F.), the lesser grain borer, on stored rough rice. Two DE commercial products, Insecto™ and Protect-It®, were applied at their respective label rates of 500 and 400 ppm to long grain rice by hand-mixing the DE with the rice. *R. dominica* were exposed for varying time intervals, mortality was assessed, and rice was held at different temperatures and relative humidities (r.h.) for 8 weeks until F_1 adult emergence. There was a significant difference in mortality between the DE treatments and untreated controls ($P<0.01$), but no significant differences with respect to the two DE products ($P\geqslant 0.05$). Mortality increased as exposure interval increased, and ranged from 15.8% to 69.2%, depending on the exposure interval. Although the general ANOVA showed a significant difference for temperature and r.h., when mortality and r.h. were compared only 5 out of 30 comparisons were significant ($P<0.05$). There was extensive progeny production in all treatments (including controls) and more progeny were produced at 32 than at 27 °C. The overall ANOVA showed a difference for treatment and r.h., but again few comparisons were significant ($P<0.05$). Results showed that the two DE products did not completely suppress *R. dominica* on rough rice, and combination treatments with another insecticide may be necessary to give complete control.

Keywords: Protectant; Inert dust; Insects; Control; *Rhyzopertha dominica*

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is one of the most important internal feeders of stored grain. Females lay eggs outside the kernels, and the newly hatched larvae bore into the kernels and develop within until they reach the adult stage (Arbogast, 1991). The mature adult bores out of the kernel and creates a large exit hole; the kernel is then referred to as an "insect-damaged kernel" (IDK). Adult *R. dominica* feed on many kinds of grains including rice, wheat, millet, and sorghum (Rees, 1995). Adult feeding on wheat causes weight loss of the kernel, and it is greatest during the first week after emergence (Gundu Rao and Wilbur, 1957). Immature *R. dominica* consume both germ and endosperm as they develop from egg to pupa, and produce more frass than both a common external feeder, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and the granary weevil, *Sitophilus granarius* (L.), another internal feeder (Campbell and Sinha, 1976).

Diatomaceous earth (DE) is obtained from sedimentary deposits in marine and fresh water systems. During previous geological periods, microscopic algae (diatoms) were able to extract silica from water, and, after they had died, the skeletal silicaceous remains were deposited and fossilized. This sediment can be mined, processed, and

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milled into fine particles for use as an insecticide for insect control in stored grains (Quarles and Winn, 1996; Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000). DE causes water loss by absorbing the cuticular lipid layer of insects, leading to death by desiccation, and the mode of action has been variously described as a physical disruption of the wax layer of the cuticle (Ebeling, 1971; Korunic, 1998; Subramanyam and Roesli, 2000). However, there are many factors affecting efficacy, including types of insect species, grain moisture, relative humidity (r.h.), temperature, and concentration of DE (Subramanyam and Roesli, 2000). Efficacy of DE generally declines with increases in r.h. or grain moisture content, and although mortality generally increases with temperature, mixed results have been reported for specific insect species and DE products (Arthur, 2000; Fields and Korunic, 2000; Vayias and Athanassiou, 2004; Athanassiou et al, 2005). The source of a particular DE can affect the efficacy, the physical characteristics of the DE itself may also be important, therefore estimates of toxicity cannot be based on geographic origin alone (McLaughlin, 1994; Korunic, 1998; Subramanyam and Roesli, 2000).

There are many commercial DE products sold in the United States, with some containing additives such as silica or pyrethrins (Subramanyam and Roesli 2000). Two common formulations are Protect-It® and Insecto™ (Fields and Korunic, 2000). Arthur and Throne (2003) found that newly-emerged adults of *Sitophilus oryzae* (L.), the rice weevil, and *Sitophilus zeamais* (Motschulsky), the maize weevil, are susceptible to the DE Protect-It®. Progeny production of these weevils was suppressed by 60–90% relative to untreated controls. Tests with newer European formulations of DE also show efficacy toward *R. dominica* on stored wheat (Kavallieratos et al., 2005; Athanassiou and Kavallieratos, 2005).

Rice is one of the most important grains for human consumption throughout the world. It is grown in the United States in only a few states, principally Arkansas, Louisiana, parts of eastern Texas and southern Missouri, and in the Sacramento valley of California (Childs, 2004). Although the rice hull offers some protection from insects, *R. dominica* can infest rough rice and cause serious damage (Breese, 1960). The kernel of rough rice consists of two modified leaves, palea and lemma, which cover the fruit or caryopsis (brown rice) (Champagne et al. 2004); thus, the efficacy of DE on wheat may be different on stored rough rice. Most of the published research with *R. dominica* and DE in the United States has been conducted on wheat or corn, and there has been little recent research with DE applied to rough rice. Therefore, the objectives of the test were to determine: (1) efficacy of two commercial formulations of DE to control *R. dominica* adults on rough rice at the labeled rates, including impacts of temperature and r.h. and (2) progeny production by *R. dominica* adults exposed on rough rice for different time intervals.

2. Materials and methods

The type of rice used in this test was XL-6 long grain rough rice, an experimental hybrid variety, at 13% moisture content as measured by a Dickey-John GAC 2000 Grain Analysis Computer (Dickey-John Corporation, Auburn, IL, USA). DE formulations were Insecto™ 90% dust (Costa Mesa, California, USA) and Protect-It® 90% dust (Mississauga, Ontario, Canada). Protect-It® contains 10% by weight of silica gel, while Insecto™ contains 90% food-grade additives. These two commercial products that were applied at their label rates of 400 and 500 ppm, respectively, specified for surface treatment to a grain mass. The rate for surface treatment was used in our test because of the comparatively small volume of rice that was to be treated, and our test was therefore more analogous to a surface treatment than a treatment to a larger grain mass. The exposure times evaluated were 1, 2, 3, 4, and 7 days at two temperatures, 27 and 32 °C and 57% and 75% (20 total combinations). These two temperatures were chosen because previous tests with wheat have shown greater production of *R. dominica* progeny at those temperatures compared to 22 °C (Arthur, 2004).

Each replicate for each DE product was treated in the following manner. Approximately 500 g of rough rice was put into a 0.95-l glass jar, the appropriate amount of DE was added, and the jar was shaken by hand for 1 min to ensure coverage of DE into the rice kernels. After mixing, the rice was subdivided into 20 individual 30-ml plastic vials containing approximately 20 g of rice each. Untreated rough rice was subdivided into 20 vials for an untreated control resulting in five replicates for each treatment, including the control.

Exposures in the individual vials were done as follows. Twenty unsexed 2-week-old adult *R. dominica* obtained from colonies maintained on rough rice at 27 °C and 60% r.h. were put in the individual vials for the various combinations for each treatment. To obtain these precise ages of adults, new cultures of these colonies were set up weekly by exposing parental adults for 5 days on ca. 350 g of rough rice in 0.95-l glass jars, then removing the adults from those jars. At rearing conditions of 27 °C and 60% r.h., it takes 6 weeks to complete the life cycle, therefore the age range of 1–2-week-old adults could be easily obtained from the colony cultures. Twelve humidity chambers were created in 26 cm × 36.5 cm × 15 cm plastic boxes, with plastic waffle-type grids at the bottom, that contained either saturated sodium bromide (NaBr) or sodium chloride (NaCl) solution below the grid for maintaining 57% and 75% r.h., respectively (Greenspan, 1977) (6 boxes for each r. h. level, 3 boxes for 27 °C and 3 boxes for 32 °C). Replicates for each treatment were grouped separately and put into separate humidity boxes, which were in turn placed inside the different temperature chambers.

Temperature and humidity inside the chambers were monitored with HOBO data recorders (Onset Computer, Bourne, MA, USA). Upon conclusion of the respective

exposure intervals, rice was sifted through a #12 sieve, and all adult *R. dominica* were removed from the appropriate vials and mortality was assessed. Insect frass and dust from feeding were put back into the vials, which were returned to the humidity chambers and the temperature incubators for another 8 weeks. After this time, the rice was sifted again, and the number of live and dead emerged F_1 adults was recorded.

Insect mortality and progeny produced after 8 weeks were analyzed using the Mixed Procedure (PROC MIXED) of the Statistical Analysis System (SAS Institute, 2001), with treatment, temperature, r.h., and exposure time as main effects. Data were further analyzed by temperature and r.h., and means for treatments were separated using the Waller–Duncan *k*-ratio *t*-test at a confidence level of $P<0.05$. Data for progeny adults were analyzed in the same manner. Lack-of-fit tests (Draper and Smith, 1981) were conducted using Table curve 2D software (Jandel Scientific, 2002) to determine maximum R^2 of any model which could be fit to the data set, the R^2 of the selected model, and the R^2 of the selected model as a percentage of the maximum R^2. Regression curves were fit to the mortality and progeny production data. This approach provides a means of accurately fitting linear and non-linear curves to biological data (Draper and Smith, 1981), and has been used in a number of previous publications (Arthur, 2000, 2001, 2004; Arthur and Throne, 2003).

3. Results

Mortality of *R. dominica* was significant at $P<0.01$ for main effects treatment ($F=886.2$; df = 2,280) r.h. (r.h., $F=23.7$; df = 1,280), temperature ($F=23.7$; df = 1,280), and exposure time ($F=29.0$; df = 4,280). Only the interactions of r.h.* treatment and temperature* r.h.* treatment were significant ($P=0.01$ and $P=0.02$, respectively), all others $P\geqslant 0.05$. There were no significant regressions with mortality as the independent variable and exposure as the independent variable in the untreated controls at either temperature or r.h. ($P>0.05$). Mortality in the untreated controls did not exceed 5% in any treatment. Mortality patterns were similar for the two DE treatments, and increased with the exposure interval (Fig. 1 A–D). Data for the two DE treatments were described by linear regression (Table 1), and the adjusted R^2 values ranged from 82.9% to 98.2%.

In the treatment combinations of 27 and 32 °C, 57% r.h., mortality of *R. dominica* gradually increased with exposure interval (Fig. 1A and B). At the 2-, 5-, and 7-day exposure intervals for each temperature, mortality was greater in the

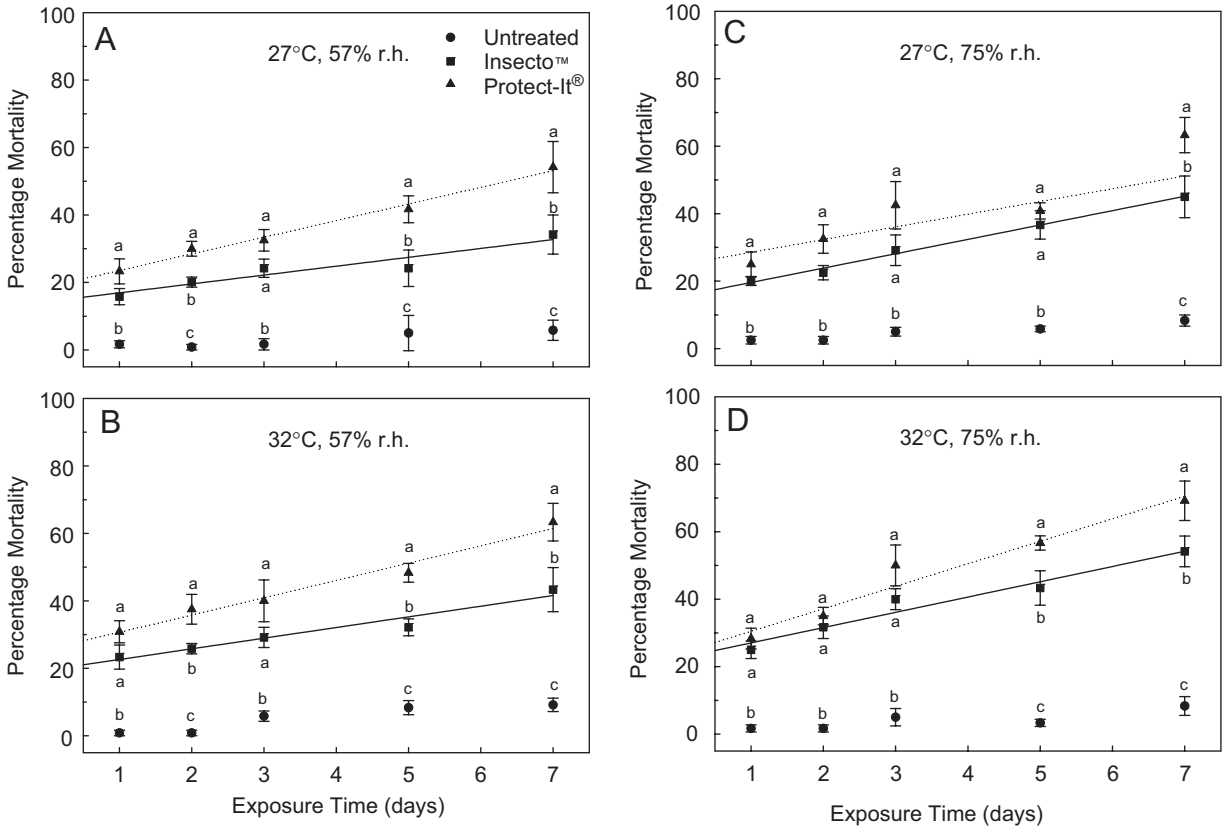

Fig. 1. Mortality of adult *R. dominica* exposed for 1–7 days on untreated rice and rice treated with 400 and 500 ppm of the commercial formulations of diatomaceous earth (DE) Protect-It® and Insecto™, respectively, and held at 57% r.h. at 27 (A) and 32 °C (B), and at 75% r.h. at 27 (C) and 32 °C (D). *Means ± SE within the same exposure interval followed by different letters for each treatment are significant at $P<0.05$ (Waller–Duncan *k*-ratio *t*-test, SAS Institute, 2001).

Table 1
Equation parameters (mean ± SE) for linear equations of the form $y = a + bx$, where y = percent mortality of *R. dominica*, $x = 1 - 7$, for day of exposure on untreated rice and rice treated 500 ppm of Insecto™ and 400 ppm of Protect-It®. Also shown in the possible maximum R^2 (Max. R^2) for any equation fit to the data, R^2 values of the linear equations, and R^2 of each equation as a % of the maximum (% Max. R^2), at two temperatures and relative humidities (r.h.)

Temperature (°C)	% r.h.	Treatment	*a*	*b*	Max. R^2	R^2	% Max. R^2
27	57	Untreated	−0.13 ± 1.6	0.9 ± 0.4	0.18	0.15	83.3
		Insecto™	14.3 ± 3.5	2.6 ± 0.8	0.31	0.28	90.3
		Protect-It®	18.6 ± 3.6	4.9 ± 0.8	0.55	0.54	98.2
	75	Untreated	1.3 ± 1.0	1.0 ± 0.2	0.39	0.36	92.3
		Insecto™	15.3 ± 3.4	4.3 ± 0.8	0.51	0.50	98.0
		Protect-It®	24.8 ± 4.1	3.8 ± 1.0	0.41	0.34	82.9
32	57	Untreated	−0.6 ± 1.4	1.5 ± 0.3	0.51	0.44	86.3
		Insecto™	19.4 ± 3.2	3.2 ± 0.7	0.40	0.38	86.3
		Protect-It®	25.6 ± 3.9	5.1 ± 0.9	0.54	0.52	96.3
	75	Untreated	0.4 ± 1.6	1.0 ± 0.4	0.26	0.19	73.1
		Insecto™	22.5 ± 3.3	4.5 ± 0.8	0.57	0.55	96.5
		Protect-It®	23.8 ± 4.4	6.7 ± 1.0	0.62	0.59	95.2

Table 2
Equation parameters (mean ± SE) for non-linear equations of the form $y = a - be - x$, where y = percent mortality of *R. dominica*, $x = 1 - 7$, for 7 days of exposure of 20 parental adults on rice treated on untreated rice and rice treated 500 ppm of Insecto™ and 400 ppm of Protect-It®. Also shown is the possible maximum R^2 (Max. R^2) for any equation fit to the data, R^2 values of the linear equations, and R^2 of each equation as a % of the maximum (% Max. R^2), at two temperatures and relative humidities (r.h.)

Temperature (°C)	% r.h.	Treatment	*a*	*b*	Max. R^2	R^2	% Max. R^2
27	57	Untreated	45.0 ± 3.0	109.1 ± 17.0	0.69	0.59	0.86
		Insecto™	34.5 ± 2.5	77.4 ± 14.0	0.63	0.52	0.82
		Protect-It	36.8 ± 2.6	80.0 ± 14.6	0.56	0.52	0.93
	75	Untreated	67.4 ± 4.1	145.1 ± 23.3	0.63	0.58	0.92
		Insecto™	54.3 ± 3.7	115.3 ± 20.8	0.65	0.52	0.80
		Protect-It®	36.8 ± 2.6	80.0 ± 14.6	0.56	0.52	0.93
32	57	Untreated	98.9 ± 4.6	188.4 ± 26.5	0.68	0.65	0.90
		Insecto™	86.2 ± 4.2	197.1 ± 23.7	0.76	0.71	0.93
		Protect-It®	80.4 ± 4.8	169.4 ± 27.3	0.59	0.57	0.97
	75	Untreated	96.5 ± 4.0	184.2 ± 22.5	0.72	0.70	0.97
		Insecto™	82.6 ± 5.1	168.3 ± 29.0	0.57	0.54	0.94
		Protect-It®	83.4 ± 5.8	162.5 ± 32.8	0.50	0.46	0.92

Protect-It® treatments than in the Insecto™ treatments, but even after 7 days of exposure, mortality was only 45.0 ± 6.2% and 50.8 ± 5.2% for each treatment, respectively. At 27 °C and 75% r.h. (Fig. 1C) and at 32 °C and 75% r.h. (Fig. 1D), there was a more gradual increase in mortality. However, there were only three instances where mortality from the DE treatments differed, in contrast to results for 57% r.h. Maximum mortality after 7 days of exposure was 69.2 ± 5.8% and 54.2 ± 4.5% for Protect-It® and Insecto™ treatments, respectively.

The general analysis showed a significant effect for both temperature and r.h. However, when mortality for individual treatments and exposure intervals were compared for differences between temperatures at the two r.h. levels, there were only two occasions (out of 15 comparisons) where there was a significant difference. Similarly, when data at each temperature were compared for differences between the two r.h. levels, there were only 3 out of 15 significant comparisons ($P < 0.05$).

Progeny production of *R. dominica* was also significant at $P < 0.01$ for main effects treatment ($F = 17.2$; df = 2,280), r.h. ($F = 341.9$; df = 1,180), temperature ($F = 12.4$; df = 1,280), and exposure time ($F = 89.8$; df = 4,280). Only the r.h.* exposure was significant ($P < 0.01$, all others $P \geqslant 0.05$). In the untreated controls and in the two DE treatments, the number of progeny seemed to plateau (Fig. 1A-D), and data were described by non-linear regressions (Table 2). Progeny production increased with exposure interval because the longer the parental adults were held on the rice, more eggs were laid. However, even though treatment was significant, it appeared to be a statistical artifact because there were only two exposure intervals where F_1 progeny production differed (Fig. 2B and C).

The effects of temperature on progeny production are shown in the equations for Fig. 2A–D in Table 2 and by comparing the number of progeny at each temperature for the two r.h. levels. More progeny were produced at 32 than

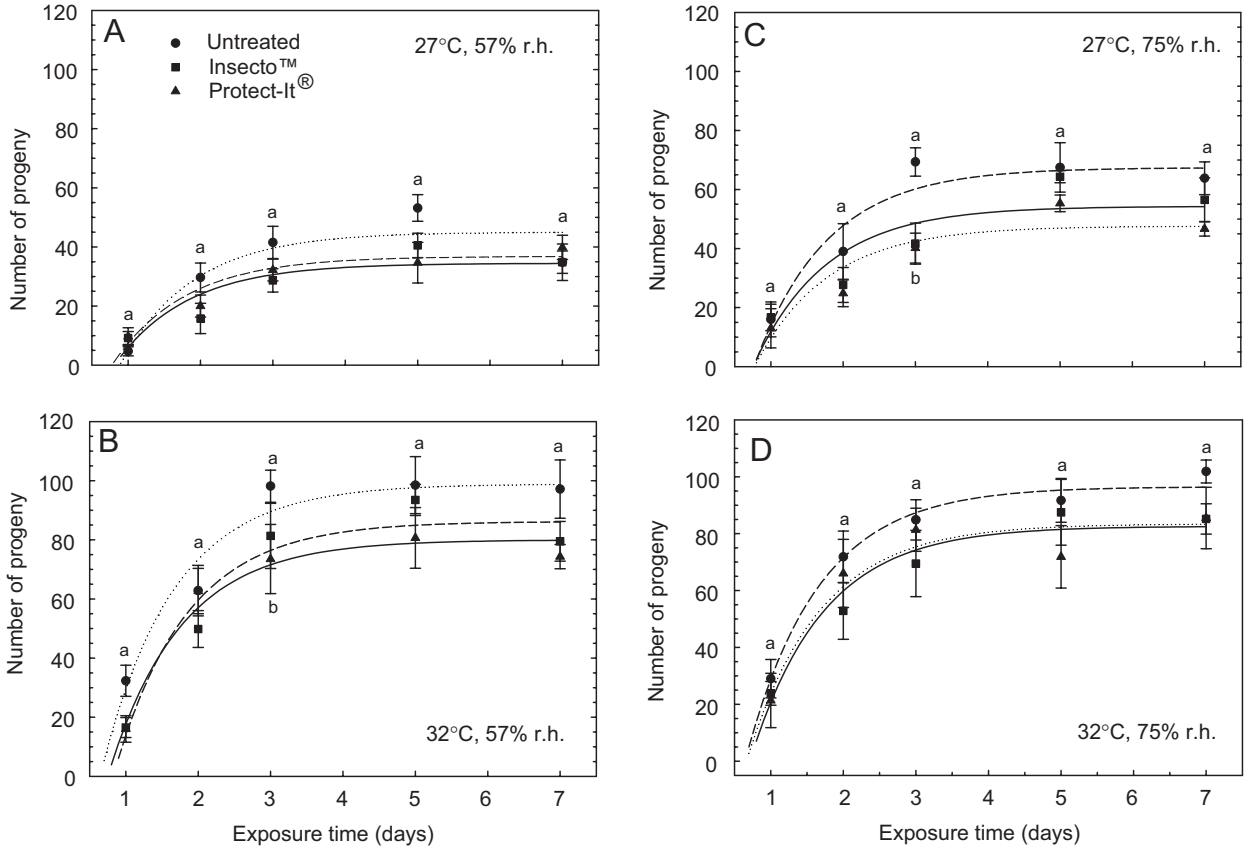

Fig. 2. Progeny adults produced from 20 unsexed adult *R. dominica* exposed for 1–7 days on untreated rice and rice treated with 400 and 500 ppm of the commercial formulations of diatomaceous earth (DE) Protect-It® and Insecto™, respectively, and held at 57% r.h. at 27 (A) and 32 °C (B), and at 75% r.h. at 27 (C) and 32°C (D). *Means ± SE within the same exposure interval followed by different letters for each treatment are significant at $P<0.05$ (Waller–Duncan *k*-ratio *t*-test, SAS Institute, 2001).

at 27 °C ($P<0.05$) in 14 of 15 comparisons at 57% r.h., but only 6 out of 15 comparisons at 75% r.h. Temperature exerted a significant positive effect on progeny production in the untreated control and in the two DE treatments. The effect of r.h. can also be seen in a comparison of progeny production. More progeny were produced at 75% than at 57% r.h. in 13 out of 15 comparisons at 27 °C, and 6 out of 15 comparisons at 32 °C ($P<0.05$).

4. Discussion

In previous tests in which *R. dominica* were exposed for 3 weeks on wheat treated with 300 ppm of Protect-It® DE, mortality ranged from 66% to 99% (Arthur, 2004). Although we used a 7-day exposure period in this test with rough rice, the application rates of the DE products were slightly higher at 400 and 500 ppm for Protect-It® and Insecto, respectively, yet mortality did not exceed 70%. In general, *R. dominica* is one of the more difficult stored-grain beetle species to control with DE (Fields and Korunic, 2000). In similar studies, *S. oryzae* (L.), the rice weevil, and *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, were exposed for various time intervals on wheat treated with 300 ppm Protect-It® (Arthur and Throne, 2003; Arthur, 2001). Mortality of *O. surinamensis* and *S. oryzae* was generally 100% after exposures of 72 and 144 h, respectively, and although some progeny production occurred in *S. oryzae*, it was far less than what was observed with *R. dominica*.

Studies by Athanassiou et al. (2005) and Athanassiou and Kavallieratos (2005) show no statistical differences in mean mortality levels of *S. oryzae* exposed on rice and wheat, and *R. dominica* exposed on rice and wheat (Kavallieratos et al., 2005) treated with European formulations of DE. However, in these studies there was generally a numerical difference of at least 20% less mortality on rice compared to wheat when *R. dominica* was exposed for 1, 2, and 7 days at concentrations of 750 or 1000 ppm of either Insecto™ or SilicoSec® DE, with narrow ranges of standard errors, yet there was no significant statistical difference. Other studies have shown evidence that DE formulations in general are less effective on rough rice than on wheat (Korunic, 1997), and similar reductions in efficacy have also been reported for some organophosphate insecticides applied on rough rice compared to wheat (Samson and Parker, 1989). The reasons for these discrepancies in mortality are not clear, but they probably related to physical and chemical composition of different grains. We visually observed rice kernels after they were treated with DE, and noted that DE dust did not give good

coverage of the rough rice husk. Normally, mortality of stored grain beetles exposed to DE decreases as grain moisture content or relative humidity increases, along with a corresponding effect on progeny production (Korunic, 1998; Subramanyam and Roesli, 2000), but this did not occur in our test. Increases in temperature also can lead to increased mortality (Arthur, 2000; Athanassiou et al., 2005), presumably because the higher temperatures lead to increased movement and the insects would pick up more of the DE particles. However, in our study we did not observe an increase in mortality with the increase in temperature.

There was a positive effect of temperature on progeny production, regardless of treatment. Previous studies on wheat have also indicated that fecundity and progeny production of *R. dominica* was greater at 32 than at 27 °C (Vardeman et al., 2006). In our test, the effect of temperature on progeny production was more evident at 57% than at 75% r.h., and the higher r.h. could have simply masked the temperature effect. This apparent increase in progeny production of *R. dominica* at 75%, in addition to the loss of efficacy at higher r.h. levels, could account for the difficulties in controlling this species with DE.

Ling et al. (1999) reported that an application rate of 700 ppm of Protect-It® was equivalent to 8 ppm of the organophosphate fenitrothion for the control of *R. dominica* on rough rice. However, even at label rates, DE can affect the physical properties of grains (Korunic et al., 1996, 1998). Combination treatments of DE + the insect growth regulator methoprene were an effective combination treatment for wheat (Arthur, 2004), which may be a more promising approach to controlling *R. dominica* on rough rice than simply increasing the application rate of DE.

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